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The effect of sodium sulfite on fiber contents of red clover when using the ANKOM® detergent fiber fractionation system

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Introduction

Several different modifications of cell wall analysis (neutral detergent fiber (NDF), acid detergent fiber (ADF) and sulphuric acid lignin (lignin (sa)) have been described and continue to be developed for forages, in particular for ruminant feeds. However, a large variation between laboratory results for fiber fractions of red clover grown in Sweden created the need to investigate the effect of specific modifications of the technique on the estimates of cell wall structure. An editorial in Animal Feed Science and Technology (2005) reviews the evolution of some overall modifications from the 60's and was used as a start for the research. In 2002 the American Organisation of Analytical Chemists approved the NDF methodology (Mertens, 2002). This article concludes that "Sixty min of refluxing at boiling temperatures achieves asymptotic extraction" and "the proposed method uses 2 additions of heat-stable amylase: one in neutral detergent (ND) solution after initiation of boiling, and one in the first residue washing step" in order to remove starch. Finally, this article states that "research by the Study Director indicated that sodium sulfite is critical for removal of proteinaceous matter in heated or cooked feeds, and it was reintroduced in the method that is being evaluated". Hintz *et al.* (1996) recommend 1:1 grams/gram of sulfite to sample, based on a series of trials. Hunt *et al.* (1995) investigated 4 neutral detergent extraction methods to determine where changes due to sodium sulfite occurred. They concluded that use of sodium sulfite in the rinse was a viable method to decrease sample analysis time without compromising NDF results. However, use of sodium sulfite is still controversial. Already in 1991, in a review of the methodology, Van Soest *et al.* (1991) stated that "Sulfite also attacks lignin and therefore should not be used in sequential analyses leading to lignin determination…"

The ANKOM^{200} Fiber Analyzer® is a machine used to determine NDF and ADF residues in 24 filter bags at a time. It has an inner chamber that can heat (100 ± 0.5°C) 2 liters of fluid and a bag suspender with trays that move vertically and rotate while maintaining a pressure of 10-25 psi. This instrument is made to be capable of creating a similar flow of solution around each sample to ensure uniformity of extraction (ANKOM^{2000} with 65 rpm agitation, ANKOM Technology). These filter bags are nitrogen and ash free, can withstand 72% sulfuric acid, and have a porosity of 25 microns. The residual material in the fiber bags is weighed after washing with ANKOM® ND solution, ANKOM® Acid Detergent Solution (AD solution) or 72% sulfuric acid and the residual expressed as % of DM or g/kg of the original DM.

On the basis of the uncertainty of 1) reflux time for NDF using the ANKOM® Fiber Analyzer, 2) the number of necessary alpha amylase additions and 3) the potential effects of sulfite on fiber fractionation, a series of trials were conducted to test the following hypotheses:

1) NDF concentration in red clover achieves an asymptote after 3 sequential washes
2) NDF and ADF concentrations in red clover are reduced with increasing doses of sodium sulfite and increasing exposure time.
3) Lignin (sa) concentration in red clover, determined sequentially after NDF and ADF determination, is reduced with increasing doses of sodium sulfite in the ND solution wash.

Materials and Methods

Five varieties of red clover (*Trifolium pratensis*: Ares, Nancy, Roseta, Taifun, and Vicky), grown at the Rådde Experimental Station, the Rural Economy and Agricultural Society, in southwest Sweden were used to test the hypotheses. The varieties were harvested June 11, 2012 from a clover variety experiment designed as a randomized block with three field blocks. The crude protein concentrations ranged from 160 to 180 g/kg DM (Nadeau *et al.*, 2014). The samples were dried and ground to pass through a 1 mm screen. Approximately 0.5 g of this material was placed in an ANKOM® "F57" filter bag. Four batches of fiber fractionation with a different treatment for each batch were analysed (first four treatments in Table 1). The same five red clover varieties were analyzed in each treatment with either 2 (SS0*3, SS20W, SS11W, SS11R) or 3 (SS0*2) replicated bags with the plant sample from each block. All samples were analyzed for NDF, ADF and lignin (sa). The results from the fifth treatment (SS02*A) were from another research project using the same samples (5 clover varieties) but including more harvest times. Only samples from same harvest and blocks were included for comparison. The SS02*A was considered the "reference" treatment as it adheres to the AOAC methodology (Mertens, 2002).

The aNDF and ADF concentrations were determined in differing batches of ANKOM®Fiber Analyzer A200 runs. The treatments used were based on the variations in methodology reported above, and were as described in Table 1.

Table 1 Abbreviations and descriptions of NDF methodology modification

Treatment name	Treatment description
SS0*3	75 minutes washing in ND solution with no addition of sulfite. Three (3) subsequent rinses with deionized water for 5 minutes each. Addition of 4 ml heat stable amylase to the wash and to the subsequent first two rinses. The bags were dried and weighed and the treatment repeated twice. The bags were thereafter analyzed for ADF and lignin (sa) sequentially.
SS20W	75 minutes washing in ND solution with addition of 20 g of sulfite in 2L ND solution. Three (3) subsequent rinses with deionized water for 5 minutes each. Addition of 4 ml heat stable amylase to the wash and to the subsequent first two rinses. This is the protocol recommended by ANKOM® (ANKOM Technology, Macedon, NY) The residual material was analyzed for ADF and lignin (sa) sequentially.
SS11W:	As S20W but with only 11 g sulfite in the ND solution. The residual material was analyzed for ADF and lignin (sa) sequentially.
SS11R:	75 minutes washing in ND solution with three subsequent rinses with deionized water of 5 min. Eleven (11) g sodium sulfite added to the first rinse. Addition of 4 ml heat stable amylase to the wash and to the subsequent first two rinses. The residual material was analyzed for ADF and lignin (sa) sequentially.
SS02*A:	75 minutes washing in ND solution with no sodium sulfite, three subsequent rinses with deionized water for 5 minutes each. Addition of 4 ml heat stable amylase to the wash and to the subsequent first rinse. The residual material was analyzed for ADF and lignin (sa) sequentially. This was considered the reference treatment.

The NDF and ADF residues were determined using the ANKOM® Fiber Analyzer A200 (ANKOM Technology, 2010). The ANKOM® DaisyII Incubator was used for lignin (sa) determination. Empty bags were included in all runs to correct for empty bag weight changes. All calculations are reported on a g/kg DM basis inclusive of residual ash (aNDF, ADF).

All statistical analyses were conducted with the R statistical software (R Development Core Team, 2013) using a linear mixed-effects models the (LMER package in R) (Bates *et al.*, 2014) with treatment as a fixed variable and feed as a random variable. The number of observations for Vicky, Nancy and Taifun in the first 4 treatments (SS0*3, SS20W, SS11W, SS11R) was: 3(varieties) × 4(treatments) ×2(blocks) × 2(bags=reps) = 48. No differences were found between blocks and effect of block was not included in the final model. The number of observations for Ares and Roseta in the first 4 treatments was: 2(varieties) × 4(treatments) ×1(block) ×2(reps) =16. The number of observations for Ares, Nancy, Taifun, and Vicky in the fifth treatment (SS0*2A) were 4(varieties) × 1(treatment) × 2(blocks) × 3(reps) =24: and for Roseta: 1(variety) × 1(treatment) × 1(block) × 3 (reps) =3. All 91 samples were included in the treatment analyses model. No differences were found between blocks and effect of block was not included in the final model. Only the aNDF concentration of the final SS0*3 treatment (third wash) was included in the statistical model for differences in treatments. Due to differences between the varieties, but the use of the same variety in each treatment, clover variety was included as a random variable in the model. Each treatment was tested against the reference treatment (SS02*A) alone and thereafter against all other treatments.

Results and Discussion

The average decrease of the aNDF concentrations from the first to the second ND solution wash (SS0*3) ranged from 11 to 13%, while the decrease from the second to the third ND solution wash ranged from 3 to 5%. The aNDF concentration did not reach an asymptote after 3 sequential washes, as can be seen in Figure 1. The reduction between second and third wash was different from zero (asymptote; $P < 0.05$). However, it should be noted that this research was undertaken by subjecting the same filter bags to repeated washes. Despite the "gentleness" of the washing and rinsing procedures, a certain continued loss might be expected through the filter bag pores, primarily due to heterogeneous particle size. More research is needed with prolonged single (non-sequential) wash times.

The sequential ND solution wash treatment (SS0*3) decreased ($P < 0.05$) NDF, ADF and lignin (sa) compared to the reference (SS02*A) treatment (Figure 2). Furthermore, the sequential ND solution treatment (SS0*3) reduced the numerical concentration of NDF, ADF and lignin (sa) more than any of the sulfite treatments (SS20W, SS11W, SS11R). Use of 11 g of sulfite in the rinse resulted in more NDF, ADF and lignin (sa) compared to the reference treatment (SS02*A) ($P<0.05$). This result was surprising and no reasonable explanation found.

The NDF concentration after SS11W was not s different ($P>0.05$) from the reference treatment (SS02*A) or 20 g sulfite in the wash (SS20W). However, a numerical decrease of NDF and lignin (sa) concentrations was seen when using 11 g of sodium sulfite in the wash, compared to both the reference treatment (SS02*A) and 20 gram in the wash treatment (SS20W). This suggests that lignin is not solubilized and lost from red clover when using

sodium sulfite in sequential NDF, ADF and lignin (sa) analyses. However, the variation between sample results (magnitude of SEM) was greater when using 20 g in the wash compared to 11 g in the wash and therefore the addition of 11 g in the ND solution wash is recommended.

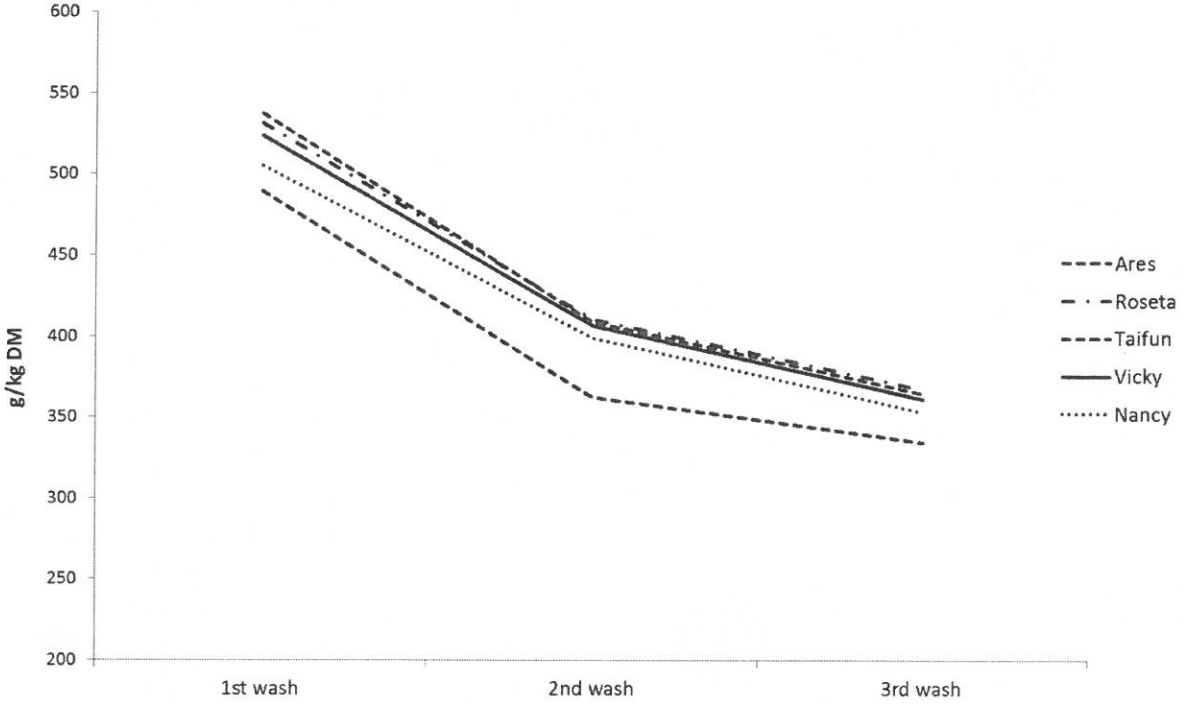

Figure 1 NDF (g/kg DM) after 3 sequential washes with neutral detergent solution.

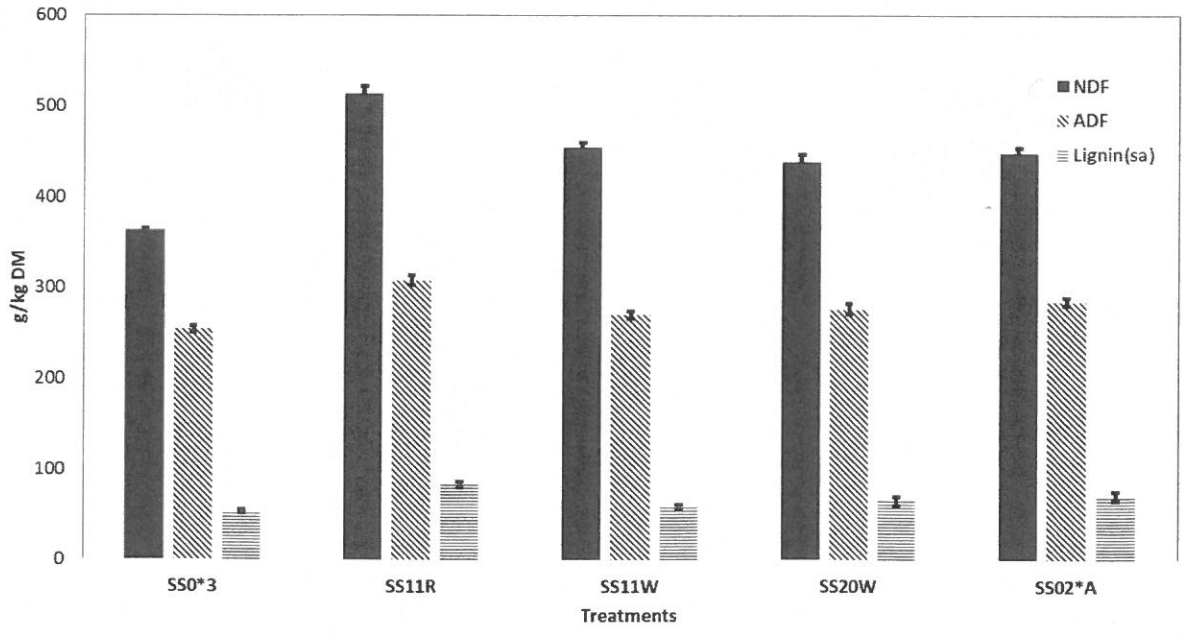

Figure 2 Comparison of NDF, ADF and lignin (sa) concentrations between different treatments. See text for treatment details and labels (SEM shown by error bars).

Conclusions

aNDF values of red clover did not reach an asymptote after three repeated analyses in the ANKOM® Fiber Analyzer. More research is needed to ascertain the correct reflux time. Repeated NDF washing affected aNDF, ADF and lignin (sa) concentrations more than addition of sodium sulfite. Use of 20 g sulfite in the wash does not produce statistically different results compared to the use of 11 g sodium sulfite for NDF, ADF and lignin (sa) values. Use of 11 g of sodium sulfite in the wash produced less variation in NDF, ADF and lignin (sa) results and is therefore recommended for red clover varieties. The ANKOM® fiber fractionation system is an extremely easy method to measure fiber. However, the differing results from varying ND solution reflux times as well as varying sodium sulfite doses and exposure times have underlined the need for more research to standardize the method in order to ensure the integrity of the plant structural components.

References

ANKOM Technology, 2010. Method 6, 4/13/11: Neutral Detergent Fiber in Feeds Filter Bag Technique (For A200, A200I). Ankom Technology, Macedon, NY, Accessed April 10, 2015 from https://ankom.com/neutral-detergent-fiber.aspx

Bates, D., Maechler, M., Bolker, B. & Walker, S., 2014. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-7, Accessed April 10, 2015 from http://CRAN.R-project.org/package=lme4.

Hintz, R. W., Mertens, D. R. & Albrecht, K. A., 1996. Effects of sodium sulfite on recovery and composition of detergent fiber and lignin. J. AOAC Int. 79, 16-22.

Hunt, J., Pinchak, W. & Hutcheson, D., 1995. Effects of incubation time and sodium sulfite upon in-vitro digestibility estimates and sample filtering time. J. Range Manage. 48, 451-454.

Nadeau, E., Hallin, O., Richardt, W., Hansen, H., Arnesson, A. & Jansson, J. 2014. Protein och fiber i rödklöver. Sid 85-88. Vallkonferens 2014. Rapport nr 18, Institutionen för växtproduktionsekologi, Sveriges lantbruksuniversitet. Konferensrapport 5-6 februari, Uppsala, Sverige, Nilsdotter-Linde, N., Bernes, G., Liljeholm, M. & Spörndly, R. (ed).

R Development Core Team, 2013. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Accessed April 10, 2015 from http://www.r-project.org

Reed, J.D., 2001. Effects of proanthocyanidins on digestion of fiber in forages. J. Range Manage. 54, 466-473.

Udén, P., Robinson, P. & Wiseman, J., 2005. Use of detergent system terminology and criteria for submission of manuscripts on new, or revised analytical methods as well as descriptive in formation on feed analysis and/or variability - Editorial. Anim. Feed Sci Technol. 118, 181-186.

Van Soest, P. J., Robertson, J. B. & Lewis, B. A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583-3597.

Van Soest, P. J. & Wine, R. H., 1967. Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. J AOAC 50, 50-55.